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INCREASED FORMALDEHYDE-INDUCED FLUORESCENCE OF EPIDERMAL MELANOCYTES IN PREGNANCY

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Abstract. Treatment with formaldehyde induces fluorescence in epidermal melanocytes of human skin. This fluorescence is probably due to dopa or dopa-containing compounds in the melanocytes. In non-pregnant women melanocytes of abdominal skin not exposed to the sun show only weak or no formaldehyde-induced fluorescence. By contrast, melanocytes from the same site in women who have just been delivered show pronounced fluorescence. This activation of melanocyte fluorescence during pregnancy is probably induced by hormones.

During pregnancy the pigmentation is increased on the nipples and the areola, on the face, in the midline of the abdomen and on the genitalia. A higher number of melanocytes as well as an increased amount of pigment of the individual cells can explain this change in pigmentation (15).

Formaldehyde treatment of skin specimens for histochemical demonstration of catecholamines and their immediate precursor 3,4-dihydroxyphenylalanine (dopa) induces a yellow-green fluorescence in the melanocytes of Caucasians (5, 6, 8), but not in those of negroes (1). The fluorescence may be due to the presence of dopa, which has been demonstrated chemically in human malignant melanin-producing cells (4, 9), but recent work also indicates that dopa peptides or thioethers of dopa may give rise to the observed fluorescence after formaldehyde exposure (11). Roentgen irradiation or exposure to short ultraviolet light increases the formaldehyde-induced fluorescence of melanocytes in Caucasians (2, 10). The genetically determined intense hyperpigmentation of negro skin is thus combined with the absence of formaldehyde-induced fluorescence of melanocytes, while the physical activation of pigment formation of weakly or moderately pigmented Caucasian skin is paralleled by an increase of the substances giving fluorescence.

It was obviously of interest to ascertain whether the pigment increase in pregnancy, probably induced by hormones (15), was correlated with changes of the formaldehyde-induced fluorescence of epidermal melanocytes.

MATERIAL AND METHODS

Ten Caucasian women who had just been delivered and 8 non-pregnant controls were studied. The hair varied in colour from very fair to dark and to the same extent in each group. The age distribution was the same in both groups.

Immediately after delivery punch biopsies were obtained in the midline just above the pubic hair border. Corresponding specimens were obtained from the non-pregnant women in different phases of the menstrual cycles. The skin specimens were immersed in propane cooled by liquid nitrogen, after which they were freeze-dried and treated with formaldehyde (7). The preparations were embedded in paraffin, serially sectioned at 10 μ and mounted for fluorescence microscopy. The light source was a high-pressure mercury lamp. The activating light was filtered through 3 mm Schott BG 12 and passed through a dark-field oil immersion condenser. The secondary filter in the tube was a Schott OG 4, 1 mm thick. The intensity of fluorescence was graded arbitrarily from 0 to 5, 0 indicating no fluorescent melanocytes, 1 just perceptible fluorescence and 5 the strongest fluorescence observed in melanocytes.

RESULTS

At the site of biopsy the pigmentation was weak in the non-pregnant women. In the women who had just been delivered the pigmentation varied widely from dark to almost imperceptible. In most of the non-pregnant women no fluorescent

Fig. 1. Weakly fluorescent epidermal melanocytes in the skin of a non-pregnant woman. × 140.

melanocytes were found. The melanocytes, when seen, were green-yellow fluorescent, unevenly distributed in the skin sections, and the number varied widely between individuals (Fig. 1). Specimens from all of the delivered women showed fluorescent melanocytes, whose fluorescence was generally stronger than in the non-pregnant women (Table I). The cells were more distinct and dendrites were more often discernible (Fig. 2).

DISCUSSION

The absence of fluorescence of melanocytes of the abdominal skin previously not exposed to the sun in the non-pregnant women parallels the weak melanin pigmentation. Tyrosinase is the enzyme that activates melanin formation by oxidation of tyrosine to dopa, which substance is then oxidized further and polymerized to melanin. Dopa is accumulated at tyrosine oxidation under *in vitro* conditions (3), and it seems probable that the increase observed in fluorescence intensity of formaldehyde-treated melanocytes indicates accumulation of dopa and/or dopa-containing compounds induced by an increased tyrosinase activity of the cells. It seems probable that tyrosinase is inactive or only weakly active in Caucasian skin that has not been exposed to the sun or stimulated to melanin formation in some other way. Therefore, no dopa or fluorescent dopa derivatives are formed. The pregnancy-induced change in the number of visible melanocytes is very marked due to the absence of any fluorescence in most of our cases under unstimulated conditions. The lowest fluorescence intensity in the delivered women was grade 2, which was the highest fluorescence intensity in the non-pregnant women, and observed in only one of them.

The finding of increased melanocyte fluorescence in the delivered women is the first direct demonstration of a probably hormone-induced change of dopa and/or dopa-containing compounds in melanocytes.

Estrogen and progesterone have been shown to induce a pigment increase similar to that observed in pregnancy in guinea-pigs (13, 14), and an increase of these hormones in pregnancy may be

Table I. *Fluorescence intensity of melanocytes of midline abdominal skin of women just delivered and of non-pregnant women*

	Fluorescence intensity				
	0	1	2	3	4
Just delivered ($n = 10$)			5	3	2
Non-pregnant ($n = 8$)	5	2	1		

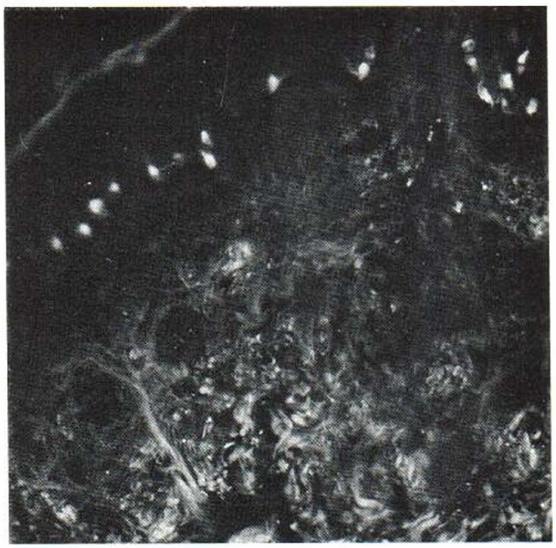

Fig. 2. Strongly fluorescent epidermal melanocytes in the skin of a woman just delivered. × 140.

largely responsible for the fluorescence changes observed by us in man. However, melanocyte-stimulating hormones of the pituitary may also be partly responsible for the increase of fluorescence observed in epidermal melanocytes during pregnancy (12).

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REFERENCES

1. Agrup, G., Falck, B., Olivecrona, H. & Rorsman, H.: Formaldehyde-induced fluorescence of epidermal melanocytes in Caucasian and negro skin. Acta Dermatovener (Stockholm) *51:* 350, 1971.
2. Agrup, G., Falck, B. & Rorsman, H.: Formaldehyde-induced fluorescence of epidermal melanocytes after a single dose of ultraviolet radiation. Acta Dermatovener (Stockholm). (In press).
3. Evans, W. C. & Raper, H. S.: The accumulation of l-3:4-dihydroxyphenylalanine in the tyrosinase-tyrosine reaction. Biochem J *31:* 2162, 1937.
4. Falck, B., Jacobsson, S., Olivecrona, H., Olsen, G., Rorsman, H. & Rosengren, E.: Determination of catecholamines, 5-hydroxytryptamine and 3,4-dihydroxyphenylalanine (dopa) in human malignant melanomas. Acta Dermatovener (Stockholm) *46:* 65, 1966.
5. Falck, B., Jacobsson, S., Olivecrona, H. & Rorsman, H.: Pigmented nevi and malignant melanomas as studied with a specific fluorescence method. Science *149:* 439, 1965.
6. — Fluorescent dopa reaction of nevi and melanomas. Arch Derm (Chicago) *94:* 363, 1966.
7. Falck, B. & Owman, C.: A detailed methodological description of the fluorescence method for the cellular demonstration of biogenic monoamines. Acta Universitatis Lundensis, Sectio II, 1965, No. 7. C. W. K. Gleerup, Sweden.
8. Falck, B. & Rorsman, H.: Observation on the adrenergic innervation of the skin. Experientia *19:* 205, 1963.
9. Falck, B., Jacobsson, S., Olivecrona, H., Rorsman, H., Rosengren, A. M. & Rosengren, E.: On the occurrence of catechol derivatives in malignant melanomas. Communications from the Department of Anatomy, University of Lund, Sweden, No. 5, 1966.
10. Olivecrona, H. & Rorsman, H.: The effect of roentgen irradiation on the specific fluorescence of epidermal melanocytes. Acta Dermatovener (Stockholm) *46:* 403, 1966.
11. Rorsman, H., Rosengren, A.-M. & Rosengren, E.: Fluorometry of a dopa peptide and its thioether. Acta Dermatovener *51:* 179, 1971.
12. Snell, R. S.: Effect of the alpha melanocyte stimulating hormone of the pituitary on mammalian epidermal melanocytes. J Invest Derm *42:* 337, 1964.
13. Snell, R. S. & Bischitz, P. G.: The effect of large doses of estrogen and estrogen and progesterone on melanin pigmentation. J Invest Derm *35:* 73, 1960.
14. — A study of the melanocytes and melanin in the skin of the immature, mature and pregnant female guinea-pig. Z Zellforsch *51:* 225, 1960.
15. — The melanocytes and melanin in human abdominal wall skin: a survey made at different ages in both sexes and during pregnancy. J Anat *97:* 361, 1963.

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